

## CLAIMS:

1. A method of protein identification, screening and/or sequencing comprising providing a library of individual proteins, one or more of which may bind to a target of interest, wherein each individual protein includes in its sequence a "barcode" sequence, which can be used to identify each individual protein in the library.
2. A method as claimed in claim 1 wherein the individual "barcode" sequences are encoded by one or more nucleic acid sequences inserted into the genes encoding the individual proteins in the library.
3. A method as claimed in claim 2 wherein the "barcode" sequence or sequences is/are flanked by one or more recognition sites for endoprotease digestion.
4. A method as claimed in claim 3 wherein the recognition site is the site for an endopeptidase such as enterokinase or Factor Xa.
5. A method as claimed in claim 3 or claim 4 wherein the library of proteins is brought into contact/association with one or more target moieties, eg target proteins.
6. A method as claimed in claim 5 wherein the proteins and one or more target moieties will bind in solution.
7. A method as claimed in claim 5 or claim 6 wherein after binding, the complexes of protein/target moiety are isolated, followed by digestion with endoprotease to release the "barcode" sequence or sequences.
8. A method as claimed in claim 7 wherein the released "barcode" sequence or sequences is/are used to design one or more synthetic oligonucleotides, eg primers, for the recovery or amplification of one or more genes encoding those proteins which bind

to the target moiety(ies).

5 9. A method as claimed in claim 8 wherein mass spectrometry is used to determine the mass of any released "barcode" sequences, which can in turn identify the released sequence or sequences, or wherein mass spectrometry is used to determine the sequence of any released "barcode" directly.

10 10. A method as claimed in any one of claims 1 to 9 wherein the library of proteins is a library of antibodies.

11. A method as claimed in claim 10 wherein the library of proteins is a library of antibody domains, eg recombinant antibody domains such as Fvs, which include antibody variable regions..

15 12. A method as claimed in claim 11 wherein the library comprises Fvs, consisting of two chains (heavy and light-chain derived chains, VH and VL).

20 13. A method as claimed in claim 12 wherein the VH and VL chains each have their own "barcode" sequence.

14. A method as claimed in any one of claims 10 to 13 wherein the "barcode" sequence is C-terminal to the Fv sequence.

25 15. A library of proteins as defined in any one of claims 1 to 14.

30 16. A method of screening a protein library comprising screening said library for one or more desired properties, followed by dereplication to identify one or more individual proteins in the library having the desired property.

17. A method as claimed in claim 16 wherein the library is screened for binding to

a target moiety.

18. A method as claimed in claim 17 wherein binding is detected by mass spectrometry, particularly matrix-assisted laser desorption/ionisation time-of-flight (MALDI-ToF) spectrometry.

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19. A method as claimed in claim 16 wherein the library is screened for a specific biological activity.

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20. A method as claimed in claim 17 or claim 18 wherein the target is a complex mixture, eg a mixture of molecules, whole cells or cell membranes.

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~~21.~~ A method of protein identification and/or sequencing comprising providing a library of individual proteins, one or more of which may bind to a target of interest, wherein each individual protein, together with its gene, is bound to an "associating moiety".

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22. A method as claimed in claim 21 wherein the library of proteins is brought into contact with the target of interest either before or after the "associating moiety".

23. A method as claimed in claim 21 or claim 22 wherein after screening for binding to the target the library is dereplicated to identify one or more proteins with a desirable property, proteins which bind to the target.

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24. A method as claimed in any one of claims 21 to 23 where the "associating moiety" is a particle.

25. A method as claimed in claim 24 wherein the particle is a latex bead.

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26. A method as claimed in any one of claims 21 to 23 wherein the

"associating moiety" is a protein or protein complex. } *mean*

27. A method as claimed in claim 26 wherein the "associating moiety" is avidin or streptavidin and each of the proteins in the library and their associated genes are biotinylated.

28. A method as claimed in claim 21 or claim 22 wherein the "associating moiety" is a bispecific binding molecule capable of binding to both the proteins and genes. } *mean*

29. A method as claimed in any one of claims 21 to 23 wherein the "associating moiety" is a living cell or cellular virus such as a bacteria or bacteriophage. → *mean*

30. A method as claimed in any one of claims 21 to 29 wherein one or other molecules which alter the properties of the proteins in the library are bound to the "associating moiety".

31. A method as claimed in any one of claims 21 to 30 wherein the genes encoding the proteins in the library are attached to the "associating moiety" prior to synthesis of the individual proteins.

32. A method as claimed in any one of claims 21 to 31 wherein the library of proteins is a library of antibody proteins, eg a library of antibody domains such as Fvs.

~~33.~~ A method of protein identification and/or sequencing comprising providing a library of individual proteins, one or more of which may bind to a target of interest, wherein each individual protein is attached to an individual "coding moiety".

34. A method as claimed in claim 33 wherein the "coding moieties" are

particles with unique identifier "codes".

35. A method as claimed in claim 34 wherein the "codes" are different ratios of measurable signal, eg fluorescent, chemiluminescent or radioactive labels, or a physical feature such as a unique marking.

36. A method for analysing mixtures of proteins comprising:

- (iii) digestion or cleavage of the protein mixture;  
(iv) fractionation of the resultant peptides; and  
(v) analysis of the resultant peptides by means of their mass and/or sequence.

37. A method as claimed in claim 36 wherein the fractionation in step (ii) is carried out using a library of protein binding agents.

38. A method as claimed in claim 36 wherein the resultant peptides are subjected to physical fractionation and/or chemical tagging as part of the fractionation step.

39. A method as claimed in claim 36 wherein the resultant peptides are subjected to addition of one or more amino acids as part of the fractionation step.

40. A method as claimed in any one of claims 37 to 39 wherein the library of protein binding agents is a library of antibodies or antibody fragments.

41. A method as claimed in any one of claims 37 to 39 wherein the protein binding agents are major histocompatibility proteins, T cell receptors and natural proteins or protein domains involved in protein-protein binding interactions, such as SH1 domains.

42. A method as claimed in claim 40 or claim 41 wherein the library of protein binding agents is pre-selected for binding to one or more proteins or peptides derived from the protein mixture or a related protein mixture under analysis.
- 5 43. A method as claimed in claim 42 wherein the protein mixture is derived from a normalised recombinant gene library.
44. A method as claimed in any one of claims 36 to 43 wherein the protein mixture is initially bound to a solid phase prior to digestion or cleavage either via the  
10 N or C-terminus or via specific amino acids or via specific sequences of amino acids.
45. A method as claimed in any one of claims 36 to 43 wherein specific amino acids or modified amino acids found in the proteins are derivatised prior to binding to a solid phase, such binding occurring either before or after digestion or  
15 cleavage of the protein mixtures.
46. A method as claimed in claim 45 wherein the specific, or modified, amino acids are derivatised with biotin prior to binding to avidin or streptavidin.
- 20 47. A method as claimed in claim 45 wherein specific, or modified, amino acids are derivatised with ligands prior to binding to ligand-specific affinity reagents.
48. A method as claimed in any one of claims 36 to 43 wherein specific naturally modified amino acids found in the proteins are bound to a solid phase using  
25 modification specific affinity reagents, such binding occurring either before or after digestion or cleavage of the protein mixtures.
49. A method as claimed in any one of claims 45 to 48 wherein more than one cycle of digestion/cleavage and derivatisation is carried out.
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50. A method as claimed in claim 49 wherein mass analysis is carried out after each cycle of digestion or cleavage.
51. A method as claimed in any one of claims 36 to 50 wherein peptides released after digestion/cleavage are fractionated using physical methods such as HPLC before or after fractionation using protein binding agents.